Mechanism of Renal Concentration of Technetium-99m Glucoheptonate

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Seventy female Sprague-Dawley rats were studied to determine the mechanism of tubular localization and the effects of commonly encountered changes in hydration and acid-base balance on renal uptake and urinary excretion of technetium-99m glucoheptonate ([^{99m}Tc]GHA). The in-vivo protein binding and protein-free plasma clearance of [^{99m}Tc]GHA also were quantitated. Twenty additional rats were studied to determine the effects of PAH competition and probenecid blockade on renal uptake of [^{99m}Tc]dimercaptosuccinic acid (DMSA) in comparison with their effects on [^{99m}Tc]GHA localization. Kidney uptake of [99mTc]GHA averaged 11.17 ± 0.49 (s.e.) % of the injected dose in control animals. This varied slightly among groups but was significantly reduced by probenecid blockade and para-aminohippuric acid (PAH) competition to 4.08 ± 0.55 (p < 0.005) and 2.39 \pm 0.14 (p < 0.005), respectively. Technetium-99m DMSA was not affected in its renal accumulation by these maneuvers. The total plasma clearance of [99mTc]GHA was lower than iodine-125 (1251) iothalamate but the clearance of the protein free supernate was higher, raising a possibility of some tubular secretion. Acidification of the urine which has been shown to reduce [99mTc]DMSA uptake appeared to have no effect on [99mTc]GHA. Hepatic uptake was minimal in all groups averaging less than 1% injected dose. These data demonstrate that renal accumulation of [99mTc]GHA is blocked by probenecid and PAH suggesting that it is actively concentrated in the proximal tubule by enzyme systems similar to those involved in PAH and hippuran transport. It appears that [99mTc]GHA uptake measures a different aspect of kidney function than [99mTc]DMSA.

J Nucl Med 26: 1308-1313, 1985

Lechnetium-99m glucoheptonate ([^{99m}Tc]GHA) has achieved extensive clinical use as a renal imaging agent although relatively little is known about its handling by the kidney. A few reports of studies of this compound have described its organ distribution in laboratory animals (1-4). Autoradiographic studies of [^{99m}Tc]GHA (5,6) demonstrate rapid clearance of most of the injected radioactivity with the major excretory pathway through the kidney. A significant fraction of the injected dose is retained for some time in the renal cortex, but reports of the exact amount vary. Approximately 20.3% of the injected dose of [^{99m}Tc]GHA has been reported to localize in Wistar male rat kidneys 1 hr after injection (1), and 13% in rabbit kidneys (2). Kieviet (3), reported significantly lower renal accumulation than other groups noting 5.6%

of the dose per g of Wistar rat kidney tissue.

Since patients referred for renal studies often have reduced renal function, as well as abnormalities of acidbase balance, it is important to evaluate the possible effects of these pathologic conditions on the excretion and renal localization of commonly used agents. The present study was designed to evaluate the effects of dehydration, osmotic diuresis, alkalosis, and acidosis as well. Renal tubular blocking agents, probenecid and PAH, and protein binding (7, 8) provide further information on the mechanism of renal accumulation and excretion of [^{99m}Tc]GHA. The results of the present study were compared with a similar investigation of [^{99m}Tc]DMSA which has been reported previously (9).

MATERIALS AND METHODS

Ninety female Sprague-Dawley rats weighing 190-220 g each were studied by the single injection clearance tech-

Received Dec. 4, 1984; revision accepted May 17, 1985.

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nique (10). After anesthetization with ether, silastic catheters (O.D. = 0.025 in., I.D. = 0.012 in.) were placed in the left femoral artery, left femoral vein and urinary bladder. The rats were restrained in plexiglass cages and allowed to awaken. Nine groups of rats were studied. Ten animals each were divided randomly into the following groups: Control (Cont) Group I, animals were given normal food ad libitum; Dehydrated (DH) Group II, animals were given food ad libitum but were not allowed access to water for 24 hr before the study; Mannitol treated (M) Group III, each rat was studied during osmotic diuresis induced with a loading dose of 250 mg of Mannitol in volume of 1 ml, followed by an infusion of solution containing 750 mg of Mannitol in 4-5 ml saline; Probenecid treated (PR) Group IV, water suspension probenecid, 100 mg per 100 g of body weight in 1 ml water (7) was administered by gastric gavage; Alkaline Urine (AK) Group V, the urine was alkalinized with 800 mg of sodium bicarbonate in 1.5 ml of distilled water by gavage; Acid Urine (AC) Group VI, the rates were allowed water, but all food was removed for 24 hr before the study, the urine acidification was achieved with 0.1 g of NH4 Cl in 1 ml of distilled water given by gavage; Para-amino-hippurate (PAH) Group VII, each rat was given PAH with a prime dose of 5 mg in 0.1 ml, followed by an infusion of 2 mg sodium PAH per ml solution (0.067 mg/min). This plasma PAH level is high enough to achieve blockade of tubular secretion. Ten additional normal control rats and ten PAH treated rats were studied after administering technetium-99m dimercaptosuccinic acid (DMSA) using the same method cited above for [99mTc]GHA. All physiologic interventions were begun one hour before the plasma clearance studies, and all of the infusions were delivered at a rate of 2 ml per hr by Sage pump and continued throughout. Renal clearance was measured simultaneously with [125] iothalamate (11) in all groups.

The [99mTc]GHA study was prepared from a commercial kit* which contained 0.7 mg GHA and 1.1 mg SnCl₂. The technetium-99m DMSA was prepared from a commercial kit[†] containing 0.55 mg/ml succinate 0.19 mg/ml anhydrous stannous chloride. The pertechnetate [technetium-99m (99mTc)] was eluted from a commercial generator. Fifteen minutes after labeling with technetium-99m tracer, 0.1 ml of [99mTc]GHA or [99mTc]DMSA (50 μ Ci) and 0.1 ml of ¹²⁵I iothalamate (10 μ Ci) were injected through the femoral vein catheter. Arterial blood samples (0.15 ml) were placed in two heparinized capillary tubes 5, 10, 20, 40, 60 and 80 min after injection and centrifuged. Duplicate 0.025 ml of aliquots were counted for 1 min in the well counter. After counting 99mTc tracer, the samples were allowed to decay and then ¹²⁵I was counted. The animals were killed immediately after the clearance studies and the distribution of the dose in the kidneys and liver was determined by counting multiple samples in a well scintillation counter. Plasma clearance and urinary accumulation of [125]iothalamate, [99mTc]GHA and

 $[^{99m}$ Tc]DMSA were plotted on semilogarithmic paper for the clearance calculation, as described previously (10). Each kidney was sliced into ten parts and counted separately. The percent dose localized in the kidneys was used for comparison between the controls and the six other groups. All results are expressed as the mean ± 1 s.e.

The determination of the protein bound fraction of the injected [^{99m}Tc]GHA was carried out using 0.025 ml of plasma, immediately after each sample collection period. The plasma protein was precipitated with 1 ml of a 10% trichloracetic acid solution, and two washings were performed. The supernate, protein free plasma and the plasma protein precipitate were counted separately. Protein free plasma clearance was calculated from the supernate.

The protein bound fraction was calculated from the bound and unbound portions. Statistical analyses were performed using t-test comparisons of sample means and standard regression analysis.

RESULTS

The total plasma clearance of $[^{99m}Tc]GHA$ was lower than $[^{125}I]$ iothalamate in controls (0.90 \pm 0.08 s.e. ml/min/100 g body weight compared with 1.47 \pm 0.06 p <



FIGURE 1

I Control, II Dehydrated, III Mannitol, IV Probenecid, V Alkaline Urine, VI Acid Urine, VII PAH, I s.e.,* p < 0.01. (\Box) [^{99m}Tc]GHA, \boxtimes [¹²⁵I]iothalamate. Plasma clearance of [^{99m}Tc]GHA and [¹²⁵I]iothalamate are shown in the bar graph. lothalamate clearance (GFR) was higher than simultaneous GHA clearance in all groups. Each group is compared against the control. GHA clearance was reduced significantly in the probenecid and acid urine groups. Dehydration, probenecid administration and urinary acidification resulted in a significant reduction in iothalamate clearance



FIGURE 2

I Control, II Dehydrated, III Mannitol, IV Probenecid, V Alkaline Urine, VI Acid Urine, VII PAH, I s.e., *p < 0.01, ** p < 0.03. (\Box) [^{99m}Tc]GHA, \boxtimes [¹²⁵I]cothalamate. Clearance of GHA and iothalamate are compared using protein free supernates of plasma. In this figure asterisk represents statistically significant difference between GHA and iothalamate clearance. In control group GHA clearance was higher than iothalamate but was not statistically significant. In dehydrated group, Mannitol, Probenecid, alkaline urine and PAH groups this difference was statistically significant

0.005) (Fig. 1) but clearance of the protein free supernate of GHA was higher than [¹²⁵I]iothalamate (1.67 \pm 0.09 compared with 1.55 \pm 0.05 P=N.S.) (Fig. 2). In the probenecid and acid urine group the GHA clearances were 0.75 ± 0.02 (p < 0.05) and 0.72 ± 0.05 p < 0.05) (Fig. 1). Forty-six precent of the [99mTc]GHA was found in the precipitated plasma 5 min after injection in the control animals. The amount of plasma protein binding of [^{99m}Tc]GHA was significantly lower in all the study groups compared with control group except alkaline urine group (Fig. 3). Plasma clearance of the protein free fraction of [99mTc]GHA correlated well with [125I]iothalamate protein free supernate clearance (r=0.82,)(Fig. 4). The total kidney uptake of [^{99m}Tc]GHA was 11.17 ± 0.49 (s.e.) % injected dose in controls. This varied slightly among groups (Table 1) but unlike [99mTc]DMSA, [99mTc]GHA concentration was markedly reduced by probenecid and PAH blockade (4.8 \pm 0.55, p < 0.0005 and 2.39 \pm 0.14, p < 0.0005). Technetium-99m DMSA renal accumulation in the control group was 42.66 ± 2.87 (s.e.) % injected dose and in PAH infused group was 40.43 \pm 1.35 (N.S.). Acidification of the urine was associated with a slight increase of renal accumulation of GHA but not significantly different from control. These findings may be contrasted with the effects of



FIGURE 3

I Control, II Dehydrated, III Mannitol, IV Probenecid, V Alkaline Urine, VI Acid Urine, VII PAH, I s.e.,* p < 0.01. (\Box) [^{99m}Tc]GHA. Relative protein binding of GHA at 5 min is shown for each group. Protein binding was high in all groups but was significantly reduced in all of physiologic alterations except urinary alkalinization

these maneuvers on glomerular filtration rate (GFR) as measured by iothalamate clearance (Table 2).

Unlike [99m Tc]DMSA, acidification of the urine appeared to have no effect on the amount of GHA in the urine (66.11 ± 2.01 injected dose compared with 67.19 ± 1.91 P=N.S.). The amount of [99m Tc]GHA in the



FIGURE 4

Y = 0.67x + 0.4, r = 0.82, p < 0.001, n = 70. Relationship between free iothalamate and free glucoheptonate clearance is shown for all groups (r = 0.82, p < 0.001)

	Item	Urine	(% injected) Kidneys	dose) Urine + kidneys	Liver	R.K./liver (% dose/g)
	Control	67.0 + 1.0	11.0 + 0.5	70 4 1 7	0.80 + 0.15	92.21 + 11.06
1.	Dehudrated	07.2 ± 1.9	12.2 ± 0.5	70.4 ± 1.7	0.09 ± 0.13	02.31 ± 11.00
11. 111	Mennitel	55.0 ± 5.1	13.0 ± 0.5	09.4 ± 5.2	0.72 ± 0.04	92.77 <u>T</u> 9.01
ш.	Mannitor	69.6 ± 2.5	11.5 ± 0.5	81.3 ± 2.4	0.09 ± 0.00	04.04 ± 7.00
IV.	Probenecid	69.3 ± 3.5	4.1 ± 0.06 [†]	73.4 <u>+</u> 3.6	0.72 ± 0.03	$27.92 \pm 3.53^{\circ}$
V.	Alkaline urine	65.4 ± 3.6	11.0 ± 0.4	76.4 ± 3.7	0.57 ± 0.03 [†]	101.82 <u>+</u> 2.90
VI.	Acid urine	66.1 ± 2.0	12.0 ± 0.5	78.1 ± 2.2	1.21 ± 0.16	49.71 ± 6.70 [‡]
VII.	PAH	76.1 ± 3.0 [†]	$2.4 \pm 0.1^{\dagger}$	78.5 ± 3.0	0.79 ± 0.7	15.63 ± 1.53 [‡]
*	= p < 0.05.					
+	= p < 0.025.					
\$	= p < 0.0005.					
+	= S.O.					

TABLE 1

urine was reduced in the dehydrated group (p < 0.05) and increased in the PAH group (p < 0.0125) (Table 1). However, the sum of urinary excretion and the cumulative concentration in the kidneys at two hours was not significantly different than the controls except in the dehydrated group (p < 0.05).

The mean urinary pH at 80 min after alkalinization and acidification was 9.03 \pm 0.08 s.e. and 4.86 \pm 0.19, respectively.

Hepatic uptake of [99mTc]GHA was minimal in all groups, averaging less than 1% of injected dose. Animals treated with alkalinization showed a decrease in liver accumulation (p < 0.05). The acid urine group showed an increase in liver accumulation but it was not statistically significant.

The marked differences between the effect of probenecid blockade and PAH infusion on renal handling of GHA and DMSA are clearly illustrated in Fig. 5.

In spite of the variations in absolute uptake induced by the physiologic alterations described, the relative uptake in each kidney remained remarkably constant and the regression relationship approached 1 (r=0.99, p < 0.001) (Fig. 6).

DISCUSSION

The mechanisms of tubular accumulation of GHA and DMSA have been previously thought to be similar, however, in the present study renal accumulation of [99mTc]GHA was blocked by probenecid and PAH suggesting that this compound is actively concentrated to a significant extent in the proximal tubule by the same enzyme system involved in PAH and hippuran transport (7, 8). As much as 80% of the renal accumulation appears to be in the proximal tubule with only a relatively small amount remaining which might be localized distally. Yee and co-workers (9) demonstrated that probenecid did not block the renal enzyme system that concentrates [^{99m}Tc]DMSA renal accumulation. Repeat studies of tubular blockade using PAH in the present study confirmed that this does not affect [99mTc]DMSA. The probability that there is a significant difference in the aspect of renal

		Plasma clearance	Kidneye			P K /liver
	Item	(ml/min/100 g)	Urine	(% injected dose)	Liver	(% dose/g)
I .	Control	1.47 ± 0.06	75.1 ± 2.9	0.22 ± 0.06	0.67 ± 0.13	1.92 ± 0.38
11.	Dehydrated	1.24 ± 0.05*	64.4 ± 6.2*	0.22 ± 0.03	0.60 ± 0.13	2.53 ± 0.57
III.	Mannitol	1.61 ± 0.06	81.4 ± 2.6*	0.27 ± 0.05	0.68 ± 0.09	2.05 ± 0.33
IV.	Probenecid	1.20 ± 0.04 [†]	76.5 ± 4.9	0.17 ± 0.01	0.69 ± 0.10	1.21 ± 0.21
V.	Alkaline urine	1.56 ± 0.09	76.8 ± 4.1	0.31 ± 0.06	0.66 ± 0.13	2.34 ± 0.28
VI.	Acid urine	1.18 ± 0.08 [†]	75.7 ± 0.1	0.52 ± 0.22*	1.35 ± 3.04 [†]	1.79 ± 0.30
<u>VII.</u>	PAH	1.34 ± 0.05	83.6 ± 2.8 [†]	0.28 ± 0.07	1.41 ± 0.25 [†]	0.90 ± 0.11 [‡]
*	= p < 0.05.					
+	= p < 0.01.					
+	= p < 0.0125.					
t	= S.O.					

TABLE 2





(\Box) Control; \boxtimes Probenecid; \boxtimes PAH, *p < 0.01, I.s.e. Effect of probenecid and PAH on renal uptake of GHA and DMSA at 2 hr are shown. Although both reduced GHA uptake significantly, neither had any significant effect on DMSA. Note that PAH and Probenecid blockade studies were done at different times with DMSA and demonstrate variability of absolute renal uptake of this compound from batch-to-batch

function measured with GHA and DMSA is supported by these data.

Acidification and alkalinization of the urine did not change either the renal concentration or urinary clearance of [^{99m}Tc]GHA suggesting that acid-base imbalance does not significantly alter [^{99m}Tc]GHA kinetics. The minimal hepatic uptake results in very high right kidney to liver ratio. This ratio of GHA renal to hepatic uptake was significantly lower in the probenecid, PAH and acid urine groups than in the control group. The alkaline urine group showed a low right kidney to liver ratio because of the relatively high liver uptake. These physiologic changes may result in higher background in renal scans and erroneous external quantification.

The marked decrease in plasma clearance of [^{99m}Tc]GHA during dehydration probably is caused by a reduction in size of the intravascular component and decreased renal perfusion. There appears to be a weak correlation with the urinary excretion, but the sum of urinary excretion and renal uptake correlates better with total plasma clearance.

Protein bound [^{99m}Tc]GHA in the plasma was 6.9% of the injected dose 5 min postinjection and 6.3% 10 min postinjection, but fell to 2.4% after 20 min. These results





N = 70, y = 1.02x - 0.04, r = 0.99. Relationship between left and right kidney uptake of GHA is shown. Although interventions used had significant effect on absolute renal uptake, they had no significant effect on the relative uptake between two kidneys (r = 0.99, p < 0.001)

support a previous report (1) of the maximal renal concentration occurring ~ 15 min after injection and rapidly decreasing and suggest that this has a significant effect on the protein bound component.

The clearance of protein free supernate was higher than the total clearance and correlated well with [¹²⁵I]iothalamate. Technetium-99m GHA clearance appears to occur by means of two mechanisms: (a) The protein bound ^{99m}Tc is excreted by tubular secretion; and (b) Protein free portions are excreted by glomerular filtration.

These data lead to several clinically important conclusions. Quantification of GHA and DMSA uptake measure significantly different renal functions. Commonly encountered abnormalities of acid base balance will significantly alter DMSA accumulation but have little or no effect on GHA. Diseases which disproportionately affect the proximal and distal tubule will lead to discordant results between GHA and DMSA uptake. Although these are major concerns, it is encouraging that relative renal uptake was the same in each kidney in all of the conditions reviewed.

FOOTNOTES

*Byck-Mallinckrodt, St. Louis, MO. *Medi-Physics Inc., Richmond, CA.

REFERENCES

 Arnold RW, Subramanian G, McAfee JG, et al: Comparison of Tc99m complexes for renal imaging. J Nucl Med 16:357-367, 1975 imaging. J Nucl Med 16:357-367, 1975

- Vanlic-Razumenic N, Malesevic M, Stefanovic LJ: Comparative chemical, biological and clinical studies of Tc99m-glucoheptonate and Tc99m-dimercaptosuccinate as used in renal scintigraphy. Nukl Med 18:40–45, 1979
- 3. Kieviet W de: Technetium radiopharmaceuticals: Chemical characterizations and tissue distribution of Tc-glucoheptonate using Tc-99m and carrier Tc-99. J Nucl Med 22:703-709, 1981
- McAfee JG, Subramanian G: Experimental model and evaluation of animal data for renal radiodiagnostic agents. In Animal Models in Radiotracer Design, Lambrecht RM, ed. New York, Springer-Verlag, p 211
- Willis KW, Martinez DA, Hedley-Whyte ET, et al: Renal localization of Tc-99m-stannous glucoheptonate and Tc99m-stannous dimercaptosuccinate in the rat by frozen section autoradiography. *Radiat Res* 69:475–488, 1977
- 6. O'Mara RE, Capps SJ, Hall JN: Changes in the distribu-

tion of short lived radionuclides in the kidney studies by autoradiography. J Nucl Med 16:554, 1975 (abstr)

- Brazeau, P. Inhibitors of tubular transport of organic compounds. In *The Pharmacologic Basis of Therapeutics*, Goodman L, Gilman A, eds. New York, MacMillan Publishing Co., Inc., 1975, pp 862-863
- Tanner GA, Isenberg MT: Secretion of para-aminohippurate by rat kidney proximal tubules. Am J Physiol 219:889-892, 1970
- Yee CA, Lee HB, Blaufox MD: Tc99m-DMSA renal uptake; Influence of biochemical and physiologic factors. J Nucl Med 22:1054-1058, 1981
- Levinsky N, Levy M: Clearance techniques. In *Handbook* of *Physiology*, Orloff J, Berliner R, eds. Washington, DC, American Physiology Society, 1973, pp 103-117
- Blaufox MD, Cohen A: Single-injection clearances of iothalamate I-131 in the rat. Am J Physiol 218:542-544, 1970